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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

21 CFR PARTS 182 AND 184

[DOCKET NO. 82N-0089]

VITAMIN D_2 AND VITAMIN D_3; PROPOSED AFFIRMATION OF GRAS STATUS, WITH SPECIFIC LIMITATIONS, AS DIRECT HUMAN FOOD INGREDIENTS

48FR 16695
4-19 83

AGENCY: Food and Drug Administration.

ACTION: Proposed rule.

SUMMARY: The Food and Drug Administration (FDA) is proposing to affirm that vitamin D_2 and vitamin D_3 are generally recognized as safe (GRAS), with specific limitations, as direct human food ingredients. The safety of these ingredients when used as food nutrients has been evaluated under the comprehensive safety review conducted by the agency. The proposal would take no action on the listing of these ingredients as GRAS substances for use in dietary supplements.

DATE: Comments by (insert date 60 days after date of publication in the FEDERAL REGISTER).

ADDRESS: Written comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

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SUPPLEMENTARY INFORMATION: FDA is conducting a comprehensive review of human food ingredients classified as GRAS or subject to a prior sanction. The agency has issued several notices and proposals (see the FEDERAL REGISTER of July 26, 1973 (38 FR 20040)) initiating this review, under which the safety of vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) has been evaluated. In accordance with the provisions of § 170.35 (21 CFR 170.35), the agency proposes to affirm the GRAS status of these ingredients, with specific limitations, when used as nutrients in conventional food [1/] and infant formula.

The GRAS status of the use of vitamin D_2 and vitamin D_3 in dietary supplements (i.e., over-the-counter vitamin preparations in forms such as capsules, tablets, liquids, wafers, etc.) is not affected by this proposal. The agency did not request consumer exposure data on dietary supplement uses when it initiated this review. Without exposure data, the agency cannot evaluate the safety of using these ingredients in dietary supplements. The use of these ingredients in dietary supplements will continue to be authorized under Subpart F of Part 182 (21 CFR Part 182).

Vitamin D is historically the antirachitic vitamin, and currently it is considered both a vitamin and a hormone. Antirachitic activity can be elicited by exposure of the skin to

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[1/] FDA is using the term "conventional food" to refer to food that would fall within any of the 43 categories listed in § 170.3(n) (21 CFR 170.3(n)).

ultraviolet irradiation, by provitamin D compounds, or by foods and other materials containing such compounds. The natural food sources of vitamin D are basically limited to fish and fish oils, eggs, liver, butter, and milk. Milk is a relatively poor source of vitamin D, unless it is fortified. Vitamin D activity is exhibited by several compounds, some of which occur naturally and some of which are synthetic. The principal compounds, and the only ones considered GRAS for addition to food, are vitamin D_2 and vitamin D_3.

Vitamin D_2 is 9,10-seco(5Z,7E,22E)-5,7,10(19), 22-ergostatetraene-3β-ol. It is produced by irradiation of ergosterol isolated from yeast and related fungi. Vitamin D_2 occurs as white, odorless crystals, melting at 115° to 118° C, with a specific rotation, $[\alpha]_D^{25°}$, between +103° to +106°. It is insoluble in water but soluble in alcohol, chloroform, ether, and fatty oils.

Vitamin D_3 is 9,10-seco(5Z,7E)-5,7, 10(19)-cholestatrien-3-ol. It occurs in, and is isolated from, fish liver oils. It is also produced naturally through sunlight activation of 7-dehydrocholesterol in the skin. Vitamin D_3 occurs as white, odorless crystals, melting between 84° and 88° C, with a specific rotation, $[\alpha]_D^{25°}$ between +105° to +112°. It is insoluble in water but soluble in alcohol, chloroform, and fatty oils.

Vitamin D_2 and vitamin D_3 were listed as GRAS nutrients in a regulation published in the FEDERAL REGISTER of November 20, 1959 (24 FR 9368). Subsequently, they were listed as GRAS nutrients and dietary supplements in a regulation published in the FEDERAL REGISTER of January 31, 1961 (26 FR 938). However, in a final rule published in the FEDERAL REGISTER of September 5, 1980 (45 FR 58837), FDA divided the nutrient and dietary supplement category into separate listings for GRAS dietary supplements and GRAS nutrients. Therefore, vitamin D_2 and vitamin D_3 currently are listed as GRAS in §§ 182.5950 and 182.5953 (21 CFR 182.5950 and 182.5953), respectively, for use in dietary supplements and in §§ 182.8950 and 182.8953 (21 CFR 182.5950 and 182.8953), respectively, for use in food as nutrients.

Vitamin D is listed in Federal standards of identity as a required ingredient in certain milk products (21 CFR 131.127, 131.130, and 131.132). It is also listed as an optional ingredient in other milk products (21 CFR Part 131), certain enriched cereal products (21 CFR Part 137), certain enriched pasta products (21 CFR Part 139), and in margarine (21 CFR 166.110). Section 412(g) of the Federal Food, Drug, and Cosmetic Act (the act) lists vitamin D as a required nutrient in infant formula, subject to level restrictions. FDA is reviewing all nutrient levels in infant formulas under a contract with the American Academy of Pediatrics. Any necessary modifications in the nutrient levels of vitamin D in infant formula will be proposed by a separate rulemaking under section 412(a)(2) of the act. Vitamin D also may be used to fortify foods as described in Part 104 (21 CFR Part 104).

In 1971, the National Academy of Sciences/National Research Council (NAS/NRC) surveyed a representative cross-section of food manufacturers to determine the specific foods in which vitamin D_2 and vitamin D_3 were used and the levels of usage. NAS/NRC combined this manufacturing information with information on consumer consumption of foods to obtain an estimate of consumer exposure to these ingredients. On the basis of these data, FDA estimates from the NAS/NRC survey that the amounts of vitamin D_2 and vitamin D_3 used in food in 1970 were 290,000 pounds and 78,000 pounds, respectively. The survey revealed that the use of vitamin D_2 in food increased approximately threefold from 1960 to 1970, whereas the use of vitamin D_3 in food was virtually unchanged during this period.

Vitamin D_2 and vitamin D_3 have been the subjects of a search of the scientific literature from 1920 to the present. The criteria used in the search were chosen to discover any articles that considered (1) chemical toxicity, (2) occupational hazards, (3) metabolism, (4) reaction products, (5) degradation products, (6) carcinogenicity, teratogenicity, or mutagenicity, (7) dose response, (8) reproductive effects, (9) histology, (10) embryology, (11) behavorial effects, (12) detection, and (13) processing. A total of 6,395 abstracts on vitamins D_2 and D_3 was reviewed, and 317 particularly pertinent reports from the literature survey have been summarized in a scientific literature review.

Information from the scientific literature review has been summarized in a report to FDA by the Select Committee on GRAS Substances (the Select Committee), which is composed of qualified scientists chosen by the Life Sciences Research Office of the Federation of American Societies for Experimental Biology (FASEB):

Absorption and excretion

All known forms of vitamin D are absorbed with food fats. Thus, essentially all factors affecting the absorption of fat in the alimentary tract affect the absorption of vitamin D. Absorption occurs in the jejunum and/or ileum, with most of the vitamin in the chylomicrons of the lymphatic system. Bile is essential for absorption of vitamin D and it also seems to be the major pathway of excretion of vitamin D_2 and vitamin D_3 after they have been metabolized to certain hydroxy derivatives.

Absorbed vitamin D circulates in the blood. Metabolites of vitamin D are formed in the liver and in the kidney; movement from the plasma chylomicrons and lipoproteins appears to occur in the liver by transfer to an α_1-globulin fraction which acts as a carrier. Kodicek has reviewed evidence to show that vitamin D is absorbed through the skin after topical application.

Metabolism

As early as 1934 Waddell found that crude cholesterol preparations contained a provitamin D constituent which, after irradiation, was more potent against rickets in chicks and hence different from that obtained by irradiating ergosterol. Since that time the multiple nature of vitamin D has been established and, in the last decade, much knowledge has accumulated concerning the metabolism of the vitamins D and their modes of action. It is apparent that vitamin D_2 and vitamin D_3 require metabolic conversion before they can function as the vitamin or hormone. The major developments involve several hydroxylated metabolites of vitamin D, with

most of the attention focused on the metabolism of vitamin D_3. After transport to the liver, a hydroxylase system in the microsomal component effects hydroxylation on carbon atom-25. Some evidence suggests that this process is feedback regulated by the concentration of the 25-OH-D_3 metabolite in the liver. A specific protein transports the 25-OH-D_3 to the kidney where further enzymatic hydroxylation occurs to yield 1,25-$(OH)_2D_3$ or 24,25-$(OH)_2D_3$, as determined by physiological circumstances. The 1,25-$(OH)_2D_3$ is 10 to 15 times more potent than vitamin D_3 when administered parenterally. It is the most potent form of vitamin D known in effecting intestinal calcium absorption, bone calcium mobilization, and elevation of serum phosphate. In response to hypocalcemia, the parathyroid glands secrete parathyroid hormone. This triggers the synthesis of 1,25-$(OH)_2D_3$, which with the parathyroid hormone, functions in the mobilization of calcium from bone and without the parathyroid hormone, effects calcium

absorption in the intestine. Such processes involve a dynamic balance in which elevated levels of serum calcium suppress secretion of parathyroid hormone, consequently decreasing the synthesis of 1,25-$(OH)_2D_3$. Thus, the latter can be regarded as a calcium metabolizing hormone as well as a vitamin.

Owing to the multi-step nature of the processes leading to the metabolic formation of 1,25-$(OH)_2D_3$, an impairment in any step can be presumed to be harmful. Diseases which may be attributed to insufficient vitamin D may in fact be due to a metabolic block in the formation of 1,25-$(OH)_2D_3$. Bone disease associated with renal failure may involve an impairment in the 1-hydroxylation process. Also, hypoparathyroidism can be linked with an impairment of 1,25-$(OH)_2D_3$ synthesis. This is indicated by the effectiveness of this vitamin D_3 metabolite in parathyroid hormone deficiency as produced through thyroparathyroidectomies in rats and occurring spontaneously in hypoparathyroid patients. For example, DeLuca

has stated, that treatment with 1 g per day of 1α,25-$(OH)_2D_3$ is entirely successful while 2,000 g of vitamin D_3 are required to be effective in treating the disease.

Minimal responsiveness to vitamin D is generally referred to as vitamin D dependency, an inherited postnatal syndrome. Alleviation of the syndrome may occur if there is continued therapy with large amounts (1.25 mg or about 50,000 IU) of vitamin D per day. The occurrence of this hereditary trait is low but the incidence appears to be higher than once suspected. The newer knowledge of vitamin D is having marked effects in the interpretation of treatment of the vitamin D-dependent, as well as normal individuals, with massive doses of vitamin D.

Progress in the study of vitamin D metabolites and the interrelationship of vitamin D to the parathyroids has been comprehensively reviewed by DeLuca, Omdahl and DeLuca, Hay and Harrison.

Animal toxicity studies

The extensive literature on the effects of vitamin D administration in many species has focused on crystalline vitamin D_2 and vitamin D_3, but also includes studies on the effects of related sterols having vitamin D activity, as well as rich sources of vitamin D, such as high potency fish liver oils.

The LD_{50} of vitamin D_2 sulfate given intraperitoneally to mice was found to be about 2,500,000 IU per kg.

Minimal dosage for production of generalized calcinosis by intramuscular injection of vitamin D_2 in male rabbits was 500,000 to 600,000 IU given in 3 equal doses at intervals of 2 days. Signs of generalized calcinosis appeared after 8 days; dosage was about 45,000 to 50,000 IU per kg per day. Calcinosis increased in severity on such a regimen if injections were extended to 3 weeks. Anorexia, loss of weight and death occurred within about 6 weeks. Vitamin D_2 given subcutaneously daily for 3 consecutive days to rabbits at a

level of 5,000 IU per kg per day, showed increased calcification of dentine but no side-effects. Administration of 10,000 IU per kg per day by the same procedure led to anorexia, weight loss and death in five to seven days. By contrast a dose of 800,000 IU of vitamin D_3 per kg daily, given subcutaneously, elicited no side-reactions. Vitamin D_2 in cottonseed oil was administered intramuscularly every other day for 30 days to 8 adult female rabbits beginning the day after observed copulation. Total dose over the 30 day period was 1.5 million IU (about 50,000 IU per kg) on each day of injection. No differences were observed between these animals and controls receiving no injected vitamin D_2 except for higher vitamin D levels in the blood of the former. Pregnant females given a total dose of 2.5 million IU (about 85,000 IU per kg per day) or more over the 30 day period died 65 days after the first injection.

In an early study performed before an IU for vitamin D had been established, groups of weanling rats were fed a stock diet containing enough irradiated yeast to supply 40 times the rickets-curative dose of vitamin D. Irradiated ergosterol was added to this diet in amounts necessary to provide vitamin D activity equivalent to 50, 1,000, 10,000, or 100,000 times the curative level. Amounts as high as 10,000 times the daily curative dose over a period of 6 months had no effect on growth and body functions. At levels 100,000 times the daily curative dose toxic effects were observed, including anorexia, labored breathing, net loss of calcium and phosphorus from the body, hypercalcemia, skeletal decalcification, and renal calcification. In more detailed studies Harris et al. fed weanling rats for 20 days on a basal diet containing irradiated ergosterol (vitamin D_2) or tuna liver oil (vitamin D_3) at a dose of about 500,000 IU per kg daily. The authors observed that this dose was at least 5,000

times the therapeutic dose for rats. There was weight loss, and calcification occurred in the kidneys, stomachs, aortas, hearts, and lungs of rats receiving either source of vitamin D. As measured by such criteria, irradiated ergosterol was more toxic than tuna liver oil.

In a study of only one source of vitamin D (electrically activated caporized ergosterol), rats fed up to 20,000 IU per kg daily for varying periods of time ranging from 100 days to 190 days showed no histological changes in the heart, lungs, liver, spleen, pancreas, stomach, adrenal glands, kidneys, aorta, or brain. Likewise, no roentgenographic or other abnormalities suggestive of toxic effects were noted for this preparation at the dose employed. Other investigators fed rats a stock diet in which the activated ergosterol (vitamin D_2) was incorporated daily during the last 2 weeks of a total study period of 4 weeks. The daily dose of vitamin D was 60,000 to 100,000 IU per kg body weight. Based on comparisons with a control group, there were no signs of harmful effects on growth rate, bone and tooth formation, tooth eruption

rates, estrus cycle, possible occurrence of tooth pulp stones, or general appearance and behavior.

There is a suggestion in the studies of Zemplenyi and Mrhova that adult male rats, fed a basal diet containing 0.7 percent cod liver oil, undergo vascular enzyme changes when they are fed, in addition, about 75,000 IU vitamin D_2 in oil daily for periods of 5 to 9 days. In the aorta there was an increase in the activity of phosphomonoesterase I and II and of 5'-nucleotidase, whereas the activity of carboxylic esterase decreased slightly. The investigators interpreted the changes in the phosphomonoesterases and nucleotidase as reflecting an increase in vascular connective tissue activity. The authors speculated that the slight change in carboxylic esterase might be related to an increase in abnormal polysaccharides or calcium salts in the aorta.

Cruess and Clark in well-controlled experiments, fed male rats daily 150,000 IU vitamin D_2 per kg body weight dissolved in sesame oil for up to 24 days. Growth was impaired, bone ash was decreased and the

organic fraction of the bones (including all the phospholipids examined) was increased. On the basis of ^{32}P uptake measurements it was apparent that hypervitaminosis D_2 increased the synthesis of phospholipids.

Hass et al. observed histologically the distribution and evolution of lesions of the vascular system in male rabbits dosed intramuscularly with 43,000 to 260,000 IU vitamin D_2 per kg body weight daily, twice weekly or thrice weekly for periods as long as 6 to 8 weeks. The amount and distribution of abnormal calcium deposits in the soft tissues increased with increasing duration and size of the vitamin D dose. The total minimal dose for production of significant generalized calcinosis was 500,000 to 600,000 IU administered over a period of 3 weeks, but the authors note that interpretation of their results was complicated by intermittent chronic purulent pyelonephritis and hepatic coccidiosis in both experimental and control animals. Calcium was most conspicuously distributed

in the aorta and its main branches, and somewhat less so in the kidneys. It was concluded that administration of vitamin D in amounts just sufficient to produce pathologic changes in 2 or 3 weeks led to mineralization of certain tissues which do not normally calcify.

Steck et al. fed adult dogs vitamin D_2 in corn oil in daily dosages of 15,000 to 500,000 IU per kg body weight. In amounts greater than 50,000 IU per kg daily the average survival time was about 12 days; with 20,000 IU or less per kg daily there was survival for indefinite periods without signs of intoxication. While weight loss, hypercalcemia, and kidney calcification were prominent in animals receiving the higher doses, these signs were absent in animals receiving 20,000 IU or less per kg body weight. Hendricks et al. concluded that the cumulative effects on tissue calcification in dogs of capsule doses of about 5,000 IU vitamin D per kg body weight per day over a period of 10 months were not as severe as a single dose of about 50,000 IU per kg body weight.

Kent et al. studied a colony of 558 monkeys (Mucaca mulatta) 3 to 9 years old, weighing 2.5 to 10 kg, that were inadvertently fed excessive amounts of calcium, phosphorus, and vitamin D (kind not stated) for about 3 months. During this period each animal received daily approximately 162,000 IU (about 16,000 to 65,000 IU per kg) vitamin D, and 3.5 g of calcium and 2.9 g of phosphorus. There was weight loss and an increase in the incidence of upper respiratory tract infections and diarrhea. Calcium and iron deposits occurred in the kidneys, salivary glands, and lungs and were found in the aortas of 12 of 34 animals dying between the 55th and the 140th day. About 1 month after termination of the dietary excesses, the surviving animals appeared to be in good health and monkeys sacrificed after 1 year showed few lesions that were considered related to hypervitaminosis D.

Comparative studies on the toxicity of different sources of vitamin D fed in excessive amounts to dogs indicated that a commercial source of vitamin D_2 had a far more rapid and severe toxic effect (calcification of soft tissues) than did equal dosage of vitamin D administered as tuna liver oil. Doses were approximately 10,000 IU per kg per day over a period of 8 to 10 months. Jung using crystalline vitamins D_2 and D_3 and irradiated ergosterol in oral doses up to 300,000 IU per kg per day in rats, found no difference in their toxicity (weight loss) but similar doses of 7-dehydrocholesterol were found to be 2 to 3 times more toxic than any of the other sources. Mrazek et al. in comparing the chronic toxicity of vitamin D_2, and vitamin D_3 and activated ergosterol in adult rats, intubated 75,000 IU per kg per day until all of the animals died. The average survival time of rats receiving vitamin D_2 was about 43 days, vitamin D_3 about 22 days, and activated ergosterol about 70 days. McChesney found that orally, vitamin D_3 is about 55 percent more toxic and dihydrotachysterol about 260 percent more toxic than vitamin D_2 in the rat. He

concluded that the relative toxicities of vitamin D_2 and D_3 correlate well with their hypercalcemic effects. Hunt et al. using daily oral doses of 50,000, 100,000 and 200,000 IU of vitamin D_2 or vitamin D_3 in rhesus monkeys found that vitamin D_3 was significantly more toxic than vitamin D_2 in this species. Animals given vitamin D_3 at all dose levels died within 160 days. Those receiving vitamin D_2 survived, developed hypercalcemia, but showed no soft tissue mineralization after sacrifice.

In a long-term study by Bills and Wirick on the effects of overdoses of vitamin D, done prior to the establishment of the International Unit, and employing approximately 1200 rats including second and third generations, it was concluded that 0.25 percent activated ergosterol added to the diet (equivalent to 100 times the minimum antirachitic level) caused no observable effect on appearance, growth, reproduction, or resistance to respiratory infections. One thousand times the antirachitic dose was found to be "perceptibly harmful," 4000 times "definitely injurious," and 40,000 times "strongly toxic."

Human toxicity studies

Of a large number of reports on the effects of excessive vitamin D intake in humans, several are noteworthy.

Steck et al. summarized their observations on 773 subjects ranging in age from 17 to 76 who were given supplemental doses upward of 3000 IU vitamin D (presumably as activated ergosterol) per kg per day for 7 days to 5 years. They concluded that the shortest period of administration producing toxicity (weight loss, increased calcium excretion) in the group receiving 3000 to 5000 IU per kg per day was 87 days. In the group receiving 6000 to 7000 IU per kg per day the shortest period for toxicity to occur was 60 days. Of the 773 subjects, about 16 percent showed toxic signs at levels up to 25,000 IU per kg per day. The adverse effects were found to be reversible even at the highest level when dosing was discontinued.

Cogan, et al. described five patients who had taken doses of vitamin D varying from 100,000 to 500,000 IU (about 1,700 to

8,300 IU per kg) daily over 2.5 months to 5 years for treatment of rheumatoid arthritis or pruritus. All patients showed hypercalcemia, band keratopathy, and evidence of renal insufficiency as indicated by elevated blood nonprotein nitrogen and inorganic phosphorus. There was no bone decalcification or elevation in serum alkaline phosphatase.

Howard and Meyer reviewed the cases of 11 adult patients age 33 to 68, who had been given daily doses of vitamin D_2 (activated ergosterol) ranging from 150,000 to 600,000 IU (about 2,500 to 10,000 IU per kg daily) as a therapeutic measure against arthritis. Fatigue, weight loss, and anorexia were observed in 2 to 18 months. The outstanding clinical signs were impairment of renal function and degenerative lesions with calcification such as band keratitis. In all cases the blood nonprotein nitrogen and serum calcium were elevated.

Chaplin et al. reviewed 111 cases of vitamin D intoxication appearing in the literature and described 7 additional cases under their observation. All seven had been

taking 50,000 to 300,000 IU (about 1,000 to 6,000 IU per kg) of vitamin D daily for periods ranging from 3 weeks to 6 years. Band keratitis, calcium-containing periarticular cysts in the vicinity of bursae, and hypercalcemia were among the characteristic findings.

DeLuca and Cozzi reported on 12 infants, ages 7 to 30 months, intoxicated by doses of 48,000 to 200,000 IU (about 10,000 to 40,000 IU per kg) daily for 30 to 50 days. The signs and symptoms observed included anorexia, restlessness, pallor, hypercalcemia, lowered alkaline reserve, and renal insufficiency.

Debré found in a clinical study of 21 cases of vitamin D overdosing in children, aged 16 months to 7 years, that a total intake of 11 to 18 million IU (time period not stated) was fatal in two instances. Patients receiving total intakes of 3 to 6 million IU (time period not stated) experienced anorexia, vomiting, thirst, headache, and joint pain. These symptoms disappeared within 10 days after vitamin D dosing was stopped.

Other effects associated with vitamin D intakes of 1,000 to 3,000 IU per kg daily for periods up to several years include confusion, lethargy, and behavioral and neuropsychiatric disturbances, manic-depressive psychosis, mental depression, general malaise, and headache, and anemia.

There appear to be no recorded cases of vitamin D toxicity from over exposure to sunlight and no reported studies of the relative toxicity of vitamin D_2 and vitamin D_3 in humans.

Linear growth of infants

The conclusion of Stearns that "the critical upper safe level for continuous intake of vitamin D must be somewhere between 800 to 1500 units daily" raises a question about adverse effects of rather modest intakes of vitamin D and therefore merits careful evaluation. The control group employed in the comparisons consisted of 36 male infants observed by Stearns et al. in a metabolic ward and fed 340 to 400 IU of vitamin D daily during all or part of

the first year of life. These infants received excellent care and presumably had little exposure to infection. Although it is acceptable to compare performance of this group with that of other groups studied under similar circumstances, growth comparisons of small groups of subjects are likely to be misleading and must be examined in detail. Unfortunately, this is not possible with respect to the linear growth of the infants studied by Stearns et al. The number of subjects available at each age was not stated and the presentations concerned semilongitudinal size data rather than incremental data. In most instances, the mean body length at various ages was presented without statistical summary of variability (e.g., confidence intervals), and statistical analysis of the differences between groups was not included.

Stearns stated, "It is emphasized that the slowing of growth noted with excessive vitamin D dosage does not appear in most infants until about five or even six months of age". She stated that 22 infants studied

by Jeans and Stearns received 1800 to 4500 IU of vitamin D daily but it is not clear how many of these infants were studied for more than 6 months. Detailed data have been published concerning only 9 of these 22 infants and 4 of the 9 received doses of vitamin D of 1800 IU or more daily only until 15 to 20 weeks of age. Thus, evaluation of the data must rest on observations of 5 infants. Charts presenting body length versus age suggest that one infant (subject G) may have had some slowing of linear growth after 26 weeks of age. Another infant (subject S) may have demonstrated some slowing after 26 weeks of age but initiation of more rapid growth appeared to precede the decrease in dosage of vitamin D. Growth of the remaining three infants did not appear remarkable. The Select Committee considers these data inadequate to support a conclusion that moderate overdosage of vitamin D (1800 to 4500 IU daily) interferes with linear growth.

Equally unimpressive as support for Stearns' conclusion are her comparisons of linear growth of the 36 infants given 350 to 400 IU of vitamin D daily while living in the metabolic unit, with data on the linear growth of larger groups of infants living at home and studied by other investigators. Because of the great differences in environment and probably in aspects of nutritional management other than intake of vitamin D, such comparisons seem inappropriate.

Fomon et al. studied three groups of male infants; one group received 350 to 550 IU vitamin D daily; a second group 1380 to 2170 IU daily; a third group 300 IU daily. The latter group was breastfed and the other two groups received evaporated milk formulas. During a test period of 168 days the rates of growth in length and weight and the serum concentration of calcium were similar in the three groups. This study failed to provide evidence that the level of overdosage of vitamin D interferes with the growth and well-being of normal infants.

Idiopathic hypercalcemia

Unexplained hypercalcemia of infants was first described in 1952 and circumstantial evidence implicating vitamin D in its pathogenesis was presented. Several reviews have helped to provide perspective on the relation between intake of vitamin D and the disorder known as "idiopathic hypercalcemia (of infancy) or infantile hypercalcemia."

The disorder is generally classified as mild or severe, although some cases cannot be easily assigned to one of these two categories because of intermediate manifestations. The best available data on incidence are from the United Kingdom where in 1960-1961 there were 35 cases per year with about 785,000 births, giving an incidence of 1 case in 20,000 live births. Because approximately 8 percent of the British cases were classified as severe, the incidence may be estimated to be 1 severe case for every 275,000 births.

Patients with the mild form demonstrate hypercalcemia and, by 3 to 7 months of age, generally demonstrate mild growth retardation. Although there are some

exceptions, long-term prognosis is generally excellent. Patients with the severe form demonstrate hypercalcemia, failure to thrive, a characteristic "elfin" facies, impairment of renal function, severe mental retardation, dense mineralization of the base of the skull and metaphyses of the long bones, and not uncommonly supravalvular aortic stenosis or peripheral pulmonary artery stenoses.

In the mid 1950s it was estimated that a substantial number of normal infants in Britain might receive as much as 4000 IU of vitamin D per day by ingesting nationally subsidized or commercially prepared milk powders, infant cereals and one of the commonly employed vitamin D supplements. According to the American Academy of Pediatrics, in 1956 the British Paediatric Association, through a survey of its members, reported that in a 30-month period from 1953-55, a total of 216 cases of infantile hypercalcemia were encountered (about 7.2 cases per month). Because of the possibility that this was related to moderate overdosage of vitamin D, drastic

reductions were advised in the amounts of vitamin D added to certain foods. By 1957, manufacturers had already reduced the extent of vitamin D supplementation. By 1959, it was believed that the old products must nearly have disappeared from the retailers and another survey of the members of the British Paediatric Association was carried out. A negligible decrease to about 6.8 cases per month was reported. However, the British Ministry of Health noted that certain vitamin supplements still provided 800 IU of vitamin D daily and steps were taken to reduce the dosage or provide a warning on the label. A third survey of the members of the British Paediatric Association was conducted in 1960-61 and demonstrated a substantial fall in prevalence of idiopathic hypercalcemia to about 3.0 cases per month. The cautious conclusion of the British Paediatric Association was as follows:

'* * * This is probably a real decrease in the prevalence of hypercalcaemia, because the methods of inquiry and the number of responding paediatricians were comparable

and the impression of most clinicians supports the quantitative data. The decrease might in fact be greater than appears if the diagnosis of hypercalcaemia has become progressively more accurate since 1953. The marked decline in hypercalcaemia does not correspond in time with the major reduction in vitamin D allowances which took place in 1957-58, but followed it after an interval of two to three years.'

Other authors seemed somewhat more convinced of the relation between reduction in intake of vitamin D and the decreased incidence of idiopathic hypercalcemia. It is to be noted that the incidence figures pertain at least primarily to the mild form of idiopathic hypercalcemia. Whether there was a decrease in incidence of the severe form in Britain in the late 1950s is unknown.

Several additional bits of information suggest the need for caution in assigning vitamin D a causative role in etiology of the mild form of idiopathic hypercalcemia. Of the 50 cases (which apparently included 4 cases of the severe form) identified by the

1960-61 survey of the British Paediatric Association, 28 percent were stated to have received no vitamin D supplements before the onset of the disease. Serum vitamin D concentrations have been found to be in the normal range in mildly affected infants and in severely affected infants have been markedly increased in some patients and normal in others. Some but not all mildly affected infants demonstrate an exacerbation of hypercalcemia when challenged with vitamin D.

There is no evidence that women whose offspring were severely affected in utero had ingested more than 400 to 800 IU of vitamin D daily during pregnancy. Furthermore, infants born to women with hyperparathyroidism do not demonstrate any of the manifestations associated with the severe form of idiopathic hypercalcemia. As summarized by the Committee on Nutrition of the American Academy of Pediatrics, if vitamin D plays a role in the intrauterine development of the severe form of infantile hypercalcemia, it must do so either by

placental transfer of the vitamin from the mother to the excessively vitamin D-sensitive fetus or, alternatively, by producing a response in the excessively sensitive mother which is deleterious to the fetus.

Vascular effects

Dalderup et al. and Knox have proposed an association between excessive vitamin D intake and death from ischemic heart disease. Lindén and Westlund reported an association between renal calculi and coronary heart disease. Lindén studied 341 men and women in Norway with disorders which qualified them for disability pensions. These included 150 persons with myocardial infarction. The disabilities included, besides myocardial infarction, angina pectoris and degenerative joint diseases. In the study were 341 controls randomly selected in the same area and of the same sex and age distribution, but without qualifying for disability pensions. Both men and women with myocardial infarction were consuming somewhat larger amounts of

vitamin D (more than 1250 IU per person per day) than any of the other groups (approximately 900 IU per person per day). There was no difference between the controls and the subjects with angina pectoris and degenerative joint diseases. The data give some support to Lindén's hypothesis that long-term ingestion of excesses of vitamin D may be a factor in the occurrence of myocardial infarction.

Kummerow et al. reported that the abdominal aorta from weanling swine fed 100,000 IU vitamin D_3 per pound of feed for 5 weeks had localized collections of fibrous and amorphous extracellular material. Smooth muscle cells appeared atypical. This did not occur in the controls on the same diet which contained 650 IU vitamin D_3 per pound of ration. The vitamin D content of the tissues of weanling swine given the large vitamin D_3 supplement for 6 weeks was several orders of magnitude higher than in the unsupplemented controls. The blood serum, muscle, fat, and liver from normal human subjects assayed for higher levels of vitamin D than these tissues from unsupplemented swine that had been fed a regular corn and soybean commercial ration.

There appear to be no studies in which an excess of vitamin D has been given in somewhat smaller doses and for longer periods of time.

Pregnancy and neonatal effects

Massive doses of vitamin D to rats have long been known to affect the estrus cycle, fertilization, and course of pregnancy. In a study of Ornoy et al. vitamin D_2 doses of the order of 4,000, 20,000, and 40,000 IU (about 10,000, 50,000 and 100,000 IU per kg, respectively) were administered daily by intubation to pregnant and nonpregnant rats. Four thousand and 20,000 units administered from the 9th day of pregnancy had no apparent deleterious influence on the fetal development. At a dose of 40,000 IU the placentas, fetuses, and bones were all small, and the progeny were nonviable. The results suggest that vitamin D_2 or a metabolite passes through the placental barrier.

In experiments with rabbits given total dosages of 1.5, 2.5, 3.5, or 4.5 million IU (about 50,000 to 150,000 IU per kg body weight on each day of intramuscular injection) of vitamin D_2 throughout the term of pregnancy (30 days) it was shown by Friedman and Roberts that the vitamin, or a metabolite, crossed the placenta. The blood levels of antirachitic substance in the mothers given vitamin D and their offspring were seven and nine times greater than in the control mothers and offspring, respectively. Also, the serum calcium levels in the offspring whose mothers received vitamin D were significantly higher than those of controls.

Nonfamilial, congenital supravalvular aortic stenosis syndrome is a concomitant of idiopathic infantile hypercalcemia. In the study of pregnant rabbits given intramuscular doses of about 25,000 IU vitamin D_2 per kg body weight throughout pregnancy, 14 abnormal aortas were noted in the 34 offspring. Six additional offspring at three months showed generalized vitamin D

vasculotoxicity, without supravascular narrowing of the aorta, of an advanced type commonly seen in the adult animal given large doses of the vitamin. None of the control animals showed any abnormalities of the aorta. Thus, this study strongly suggests that large doses of vitamin D during pregnancy have an effect on the fetus and the excessive vitamin, or a metabolite, may be responsible for supravalvular aortic stenosis.

In related studies by Friedman and Friedman and Mills in rabbits, relationships were found between vitamin D excess in pregnancy and certain craniofacial and dental anomalies of the supravalvular aortic stenosis syndrome. Fifteen pregnant rabbits were given divided doses of vitamin D_2 intramuscularly in cottonseed oil every other day, starting on the second day after insemination and continuing until delivery. The total dose administered was 750,000 IU, an average of approximately 27,000 IU (about 13,000 IU per kg body weight) per day. The offspring from the vitamin D-treated mothers

had hypoplasia of the mandible, congenital absence of teeth, microdontia, and enamel hypoplasia. The most marked functional accompaniment of these abnormalities was severe malocclusion of the teeth. Many of the test animals had peculiar facies, premature closure of the cranial bones, strabismus, odd-shaped ears, and low birth weight.

In the interpretation of experiments based on the use of massive oral doses (e.g. 50,000-100,000 IU per kg daily to rats during the last 9 days of pregnancy) of vitamin D it should be recognized that such amounts reportedly not only damage the placenta but also alter the normal physiologic transfer mechanisms of the placental barrier. However, such effects do not invalidate the experiments as a whole. Haddad et al. have shown that the placental transfer for vitamin D_3 and its active hydroxylated metabolite proceeds at comparable rates. Also, the data suggest that vitamin D_3 metabolites cross the placenta rapidly and/or are produced by the fetuses. The findings leave room for the

possibility that the fetus is capable of transforming vitamin D_3 from the mother to the metabolite(s) observed in the fetal tissue. The data support the suggestion made in 1967 that idiopathic hypercalcemia and supravalvular aortic stenosis of infants might be the consequence of a vitamin D metabolite produced in maternal or placental tissues during pregnancy.

The variability between individuals and genetic strains in the response to maternal excesses of vitamin D has been shown in various studies. The findings of Ornoy et al. illustrate such variability. Wistar strain rats were given 40,000 IU (about 200,000 IU per kg) ergocalciferol in olive oil daily by intubation from the tenth to twenty-first day of pregnancy. Charles River rats (a more susceptible strain) were similarly dosed with 40,000 IU and a separate group was given 20,000 IU of the vitamin. The young of all Charles River rats died even at the smaller dose level. Seven of the litters of 14 Wistar rats given 40,000 IU of vitamin D per day survived beyond the first postnatal day. The

surviving offspring showed no gross malformation at birth but after a few days skeletal deformities developed. It was concluded that the postnatal effects at these high dosage levels were not due to persistence of an excess of vitamin D in the tissues or through the milk, but resulted from a fundamental teratogenic effect on the fetal osteogenic tissues which became manifest as the bone developed. In the same laboratory it was reported that toward the end of pregnancy in Wistar rats receiving oral doses of 20,000 to 40,000 IU of vitamin D_2 daily from early in the second week of gestation, the chorioallantoic placentae showed a delay in trophoblastic maturation and blood vessel formation in the villi. Calcification was detected around fetal and maternal blood vessels.

Mutagenicity

The Select Committee is not aware of any experimental data concerning the mutagenic activity of vitamin D that have significant bearing on the health aspects of vitamin D as a food ingredient.

Carcinogenicity

Barry et al. applied 0.3 percent ergosterol and calciferol in benzene twice weekly to the skin of mice and found no tumors (epitheliomas, papillomas) after 600 days. Jones et al. considered whether vitamin D-induced hypercalcemia might affect the incidence of tumor metastases in rats. Normal and thyroparathyroidectomized Sprague-Dawley rats were given subcutaneous injections of 20,000 IU of water-soluble vitamin D_3 (about 130,000 IU per kg per day) on alternate days for one week. The animals were then given injections of Walker sarcoma cells. Seventeen days following the tumor inoculations the animals were sacrificed and examined for the presence of gross tumors in the liver and mesentery. Hypercalcemia occurred in all animals but there was no change in the incidence of tumor takes in the animals given vitamin D_3 as compared to controls. Questions concerning the possible carcinogenicity of large amounts of vitamin D were reviewed in 1950 by Touraine and Zureick who concluded that the evidence was negative. However,

they suggested that further study in animals and accumulation of data from clinical experiences and observations were warranted.

Interaction with drugs and vitamin A

The biologic action of vitamin D_2 and vitamin D_3 requires several metabolic steps, some of which include protein synthesis. Thus, drugs and related substances which affect any of the metabolic steps also affect the manifestation of activity from ingested vitamin D. For example, actinomycin D and cycloheximide, well known for their effects in certain metabolic pathways, inhibit the functioning of $1,25\text{-}(OH)_2$ vitamin D_3.

Several authors have reported an increased incidence of rickets in children regularly treated with multiple doses of anticonvulsant drugs; in the adult the interaction may lead to hypocalcemia or osteomalacia. The abnormalities reported are very similar to those observed in vitamin D deficiency. Hypophosphatemia, lowered concentrations of circulating vitamin D metabolites, increase in alkaline

phosphatase, lowered intestinal absorption of calcium, and lowered bone mass have also been reported. Richens and Rowe reported pheneturide, primidone, phenytoin and phenobarbital as the anticonvulsant drugs most commonly associated with hypocalcemia. Vitamin D supplementation was effective in the treatment of both rachitic and osteomalacic changes in bone. These observations led to the study of the effects of phenytoin (Dilantin®) on the metabolism of the labeled vitamin D_3 and 25-(OH)-vitamin D_3. Short-term experiments in rats showed that both vitamin D_3 and its metabolite disappeared more rapidly from the serum of phenytoin-treated animals than of controls. Villareale *et al.* studied interactions between vitamin D_3 and phenytoin in chicks and concluded that phenytoin acts on the metabolism of vitamin D_3 or on tissue responses to the vitamin. Gascon-Barré and Côté have shown that a 21-day pretreatment of rats with phenobarbital or phenobarbital-phenytoin, but not phenytoin alone, increased the LD_{50}

of acutely administered vitamin D_3; the median time to effect was also prolonged. Both of these findings are consistent with an effect of these anticonvulsants on vitamin D_3 metabolism. Gascon-Barré and Glorieux have shown that the total excretion of tritiated 25-(OH)-vitamin D_3 is greater in phenobarbital-treated animals than in controls.

The interrelationships between combined hypervitaminosis A and D were explored by Clark and Bassett. Young and adult rats were intubated with vitamin D_2 and vitamin A palmitate in sesame oil. In animals dosed daily with 18,000 IU vitamin D_2 (about 180,000 IU per kg) and 30,000 IU vitamin A for 60 days the growth rate and survival rate were better than in other rats receiving the same amount of vitamin D and 3,000 IU of vitamin A or less, and there was less calcification in the soft tissues and less osteolytic action in the animals given the higher dose of vitamin A. There were no animals given vitamin A without large amounts of vitamin D. In a study by Taylor et al. day-old chicks were fed for 4 weeks

on diets containing 4 levels of vitamin D and vitamin A (1, 10, 100, and 1000 times the basal level in all 16 combinations), with the object of investigating a possible antagonism between the two vitamins. At level 1 the chicks were consuming, at the start of the experiment, about 750 IU of vitamin D per kg body weight per day and at level 1000, about 750,000 IU per kg per day. Only diets containing 1000 times the basal level of one or both vitamins depressed growth and induced significant changes in blood calcium, inorganic phosphate and acid phosphatase.

Views of official bodies

In 1963 the Committee on Nutrition of the American Academy of Pediatrics reviewed the relevant literature on vitamin D and issued a statement of policy. In part, the Committee concluded that because of the prevalent practice of food fortification in the United States and Canada, there was a definite possibility that the individual, even the young infant, may ingest considerably more than the recommended vitamin D allowance, and intakes of 2,000 or

3,500 IU per day were possible, particularly beyond infancy. The Committee also pointed out that although there has been no specific evidence that intakes of this order produce deleterious effects beyond infancy, the long-term consequences of this new nutritional situation on older children or adults are entirely unknown. The Committee stated that the practice of enriching foods other than milk and infant formula products is not justified, and discontinuation of fortifying other foods was recommended.

In 1967 a review and policy statement on vitamin D were prepared by Fraser in consultation with the Committee on Nutrition of the American Academy of Pediatrics and endorsed by it. It was concluded that the evidence concerning infantile hypercalcemia did not alone provide justification for extensive change in national policies relating to vitamin D. However, it was recommended that there be sensible moderation in consumption of vitamin D at all ages because the potential toxicity of vitamin D after long-term intakes, that exceed requirements by several orders of magnitude, was still unknown.

In 1973 the Food and Nutrition Board of the National Academy of Sciences issued a policy statement, superseding theirs of 1968, on improvement of the quality of foods. The Board continued its endorsement of the enrichment, fortification, and restoration of the nutritional value of certain foods, including the addition of vitamin D to milk, fluid skim milk and nonfat dry milk.

In 1975 the Committee on Nutritional Misinformation of the Food and Nutrition Board issued a report which included the conclusion that "excessive amounts" of vitamin D are hazardous and only individuals with diseases affecting vitamin D absorption or metabolism require more than 400 IU per day; such needs should be established by clinical evaluation, and treatment should be specifically recommended and supervised by physicians.

It is to be noted that in 1977 the Food and Drug Administration proposed deletion of the provision for use of vitamin D as an optional ingredient in enriched rice, pointing out that such addition would serve only to increase the excessive levels of intake of the vitamin. (Ref. 1.)

The Select Committee has carefully evaluated all the available safety information on vitamins D_2 and D_3. In the Select Committee's opinion:

> In the absence of adequate exposure to sunlight or equivalent light, dietary intake of vitamin D is required for maintenance of health. Vitamin D occurs naturally in fish and fish oils, eggs, liver and dairy products. The amounts naturally present in dairy products are generally inadequate to meet the requirement and vitamin D_3 is added to evaporated milk, infant formulas and to most fresh fluid cow milk sold by dairies. In addition, vitamin D_2 or vitamin D_3 is commonly added to margarines, to certain breakfast cereals and to a few other foods. Fortification of milk with vitamin D_3 since the 1920s has been credited with the marked reduction in incidence of rickets.
>
> The estimated requirement for vitamin D in the absence of exposure to ultraviolet light is believed to be 100 to 200 IU per day and the Recommended Dietary Allowance of the Food and Nutrition Board, National Research Council, is 400 IU per day. From food sources of vitamin D (naturally

occurring or added) it is unlikely that an infant would receive more than 1000 IU per day (perhaps 200 IU per kg per day), a preschool child more than 2000 IU per day (less than 200 IU per kg per day) or an adult more than 5000 IU per day (less than 100 IU per kg per day). However, better estimates of current intakes of vitamin D from dietary sources should, in due course, be developed.

Unequivocal manifestations of vitamin D toxicity including vascular effects have not been reported from consumption of foods including foods fortified with the vitamin. Observations on patients undergoing vitamin D therapy have shown that intakes of vitamin D 1000 IU per kg per day or more (at least 60,000 IU per day for a 60 kg adult) have in some instances been associated with evidence of toxicity. Studies of individuals with disorders qualifying them for disability pensions suggest that long-term ingestion of vitamin D in excess of 1000 IU per day may be a factor in the occurrence of myocardial infarction. However, for the adult there is relatively little likelihood of consumption

of such amounts of vitamin D from that currently added to food.

The only suggestions that toxic effects may be produced by intakes of vitamin D less than 1000 IU per kg per day concern two special problems of infants: (1) such intakes may interfere with linear growth, and (2) the rare disorder, idiopathic hypercalcemia, may be caused by or aggravated by such intakes. For reasons detailed in the body of this report, the Select Committee finds unconvincing the few reports which attribute interference in linear growth to intakes of vitamin D less than 1000 IU per kg per day. The incidence of idiopathic hypercalcemia is estimated to be 1 in 20,000 births for all forms of the disorder and 1 in 275,000 births for the severe form. This incidence is low and must be considered in relation to the demonstrated desirability of fortifying foods with vitamin D. The Committee recognizes also that vitamin D intake may aggravate the manifestations of idiopathic hypercalcemia but considers it unlikely that there is a causal relationship.

> At the same time, it is evident that the margin of safety between intakes currently achieved by some infants from all sources (perhaps 200 IU per kg per day) and the amounts (1000 to 3000 IU per kg per day) that may produce toxic manifestations in otherwise normal infants is relatively low. There is strikingly little information on the effects of moderate overdoses of vitamin D, particularly in the range of about 200 to 3000 IU per kg per day for all age groups. Additional data are needed for evaluation of the safety of vitamin D in this dosage range. Moreover, evaluations should recognize that relatively long periods are required for adverse effects of vitamin D to become recognizable. Thus, there appears to be a need to limit intakes of vitamin D from all sources, including food and vitamin preparations (Ref. 2).

The Select Committee concludes that no evidence in available information on vitamin D_2 and vitamin D_3 demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used in food at levels that are now current and in the manner now practiced. However, the Select Committee also states that it is not possible to determine, without additional data, whether a significant increase in consumption of these substances would constitute a dietary hazard (Ref. 3).

FDA has undertaken its own evaluation of the available information and, insofar as vitamin D_2 and vitamin D_3 are used as nutrients in conventional foods, agrees with the conclusion of the Select Committee. Based upon available safety data, the agency concludes that vitamin D_2 and vitamin 3 should be affirmed as GRAS with specific limitation placed upon their use. FDA shares the Select Committee's concern that the margin of safety for infants appears to be relatively low.

FDA has evaluated the available data and estimates that the levels of use of vitamin D proposed in this document will result in an average lifetime exposure of about 200 to 300 international units per day (IU/day) for individuals between the ages of 2 to 65 (Ref. 4). This estimate is an approximation because it represents a daily average lifetime exposure and does not address short-term fluctuations in eating habits that could result in significantly higher or lower consumption. This estimate also does not address the potentially elevated consumption of vitamin D by young children over a period of several years during childhood.

The Select Committee estimates that daily consumption of vitamin D from food sources (both added and naturally occurring) is unlikely to exceed 1,000 IU/day for infants or 5,000 IU/day for adults. FDA believes that these estimates by the Select Committee represent reasonable upper limits on daily consumption, and that day-to-day fluctuations in diet are unlikely to result in

higher consumption levels. However, as discussed above, the agency's estimate represents likely average lifetime consumption levels. Therefore, FDA believes that these two sets of estimates are neither incompatible nor mutually exclusive.

FDA ordinarily bases its GRAS affirmation proposals on consumption information compiled in the 1971 NAS/NRC survey of food manufacturers. However, the Select Committee indicates in its conclusion that the NAS/NRC data on the amount of vitamin D_2 and vitamin D_3 used annually in the United States lead to consumption estimates that are unrealistically high. The agency agrees with this assessment but also believes that the NAS/NRC survey information should not be dismissed without further consideration. In an attempt to clarify this issue, FDA requested that NAS/NRC reevaluate its survey information to identify potential sources of error. NAS/NRC responded that its survey information on vitamin D_2 and vitamin D_3 probably contained inaccuracies such as failure to account for dilution of multiple strength solutions and failure to differentiate between use in human food, in animal feed, or in dietary supplements (Ref. 6).

FDA believes this explanation of the NAS/NRC survey data is plausible but not conclusive. Dr. A. W. Norman (Ref. 5) noted that industrial preparation of vitamin D produces several chemically similar compounds that are difficult to separate, and that, consequently, vitamin D is marketed as an impure resin rather than as the crystalline material, at least for use in animal feeds. The agency is concerned that food processors may be using this impure resin as if it were pure crystalline vitamin D_2 or D_3 and that this practice may have contributed to the high estimates of vitamin D usage in the NAS/NRC report. Therefore, FDA requests additional information on the levels of vitamin D added to food, and information on whether the impure resin is used in food. The agency notes, however, that such a resin does not conform to the specifications for food-grade vitamin D_2 or D_3 presented in the Food Chemicals Codex, 3d Ed. (1981), and is not included in these proposed regulations.

The agency also is concerned about the magnitude of the overages of vitamin D (i.e., levels in excess of those stated on the nutritional label) that might commonly be added to certain foods to offset losses during processing or storage. This concern is based on information that overages are a common practice in the manufacture of fortified foods, as indicated by the Committee on Nutrition of the American Academy of Pediatrics (Ref. 7). FDA notes that regulations governing the addition of

vitamin D to standardized foods do not permit overages of this vitamin beyond the limitations of current good manufacturing practice. Therefore, the agency requests that information on the overages of vitamin D added to foods be submitted as comments on this proposal.

Regarding the cardiovascular toxicity of vitamin D, the new study of Peng, et al. (Ref. 8), reported the appearance of signs of arteriosclerosis in squirrel monkeys fed vitamin D_3, with or without high levels of cholesterol, for 10 to 18 months. The most significant effect, appearance of elevated atheromatous plaques, was observed in animals fed a combination of 500 IU vitamin D_3 plus 0.5 percent cholesterol daily. Animals fed 1,000 IU/day of vitamin D_3 (without cholesterol) showed slightly increased intimal thickening when observed under light microscopy. Animals fed 500 IU/day of vitamin D_3 (without cholesterol) showed cellular changes that were observable only under the electron microscope.

FDA tentatively has concluded that the results of this study provide an insufficient basis for altering FASEB's conclusions regarding the safety of vitamin D because of the small number of animals used, and because no information was presented on the purity of the vitamin D_3 used in the study. Additionally, no independent corroboration of these results has been reported by other investigators. However, FDA is aware of concerns raised by previous studies that have suggested links in humans between vitamin D and hypercholesterolemia (Ref. 9) and

between vitamin D and myocardial infarction (Ref. 10). The agency notes that similar concerns have been expressed editorially by Peng and Taylor (Ref. 11).

FDA has carefully evaluated two recent short papers which were not reviewed by the Select Committee but which purport to demonstrate a relationship between vitamin D_2 administration and the appearance of mammary tumors in mice (Refs. 12 and 13). The first study (Ref. 12) investigated directly the carcinogenic activity of vitamin D_2 in the mammary gland of mammary tumor virus-infected mice. The study purports to show the effect on the latency period. However, it is a preliminary study with technical deficiencies and is unsubstantiated by further work. The second study (Ref. 13) indicated that vitamin D_2 may have some weak estrogenic activity. However, no tumors were reported, and FDA concludes it is not valid to consider vitamin D_2 a carcinogen based upon a potential weak estrogenic activity. Therefore, the agency has concluded that it is inappropriate to take any regulatory action based on these two studies. Although the Select Committee did not specifically address the issue of carcinogenicity in its opinion on the safety of vitamin D_2 and vitamin D_3, it did cite the results of earlier work that indicated that studies of the carcinogenicity of vitamin D were negative. Although FDA encourages further

study of this problem, and solicits any additional information that may be available in response to this proposal, the agency believes that the results of these two studies, as well as those reviewed by the Select Committee, do not provide evidence that vitamin D is a carcinogen.

In view of FDA's and the Select Committee's agreement that limitations on vitamin D consumption are warranted, FDA is interested in maintaining an up-to-date knowledge of the consumption, purity, and toxicity of vitamin D, including information on studies in progress. Therefore, FDA requests comments and additional information from industry, the scientific community, and all other interested parties to supplement the agency's knowledge concerning these matters.

FDA estimates that typical exposures to vitamin D from addition of this ingredient to conventional foods at the levels proposed by this document are about 200 to 300 IU/day for an adult and about 300 to 400 IU/day for an infant (Ref. 4). The agency concludes that these levels of use represent current good manufacturing practice, are safe, and may be affirmed as GRAS.

The food categories in which use of vitamin D is being proposed for affirmation as GRAS are primarily those that were reported in the NAS/NRC Phase II survey. However, FDA is unaware of any current use of vitamin D in nonalcoholic beverages or beverage bases other than in milk and in infant formula, even though such use was reported in that survey. Therefore, FDA has not included this food category in this proposal. In addition, the agency believes that uses of vitamin D in sweet sauces, as

reported in the NAS/NRC survey, refer to products used as flavorings for milk, and these uses have therefore been included in the "milk products" category. The agency solicits comments on these and other uses of vitamin D_2 and vitamin D_3 in foods.

FDA recognizes that dietary supplement preparations can be a significant source of dietary vitamin D in addition to that that is added to conventional foods. FDA shares the Select Committee's concern that daily intake of vitamin D from all sources may be approaching potentially toxic levels, especially for small children. Based on FDA's estimate of consumption for vitamin D in food, the daily consumption of a multiple vitamin tablet that contains the recommended daily allowance of vitamin D (400 IU/day) would approximately double the exposure of a child. A separate report written by Dr. Herman I. Chinn of the

Life Sciences Research Office of FASEB, completed at the request of FDA, expressed a similar concern (Ref. 14). FDA's concern about the levels of vitamin D consumed has been heightened by a recent report of toxicity in two small children given high doses of vitamins A and D (Ref. 15). The two cases involved in this report appear to have resulted from excessive use of dietary supplements and also appear to be isolated incidents. FDA is unaware of any widespread hazard associated with vitamin D in dietary supplements. However, because the NAS/NRC survey did not specifically request use data on dietary supplement uses, FDA does not have adequate data upon which to judge the exposure to vitamin D_2 and vitamin D_3 resulting from their use as dietary supplements. Without such exposure data, the agency cannot evaluate the safety of the use of these ingredients in dietary supplements and, therefore, can take no action at this time on the GRAS status of vitamin D_2 and vitamin D_3 for this use. Therefore, FDA is taking no action on the listing of these ingredients in Subpart F of Part 182 as dietary supplements.

Copies of the scientific literature review on vitamin D, the report of the Select Committee on vitamin D_2 and D_3, and the mutagenicity evaluations of vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) are available for review at the Dockets Management Branch (address above) and may be purchased from the National Technical Information Service, 5285 Port Royal Rd., Springfield, VA 22161, as follows:

Title	Order no.	Price code	Price*
Vitamin D_2 and D_3 (Select Committee report)	PB293-099/AS	A03	$ 7.50
Vitamin D (scientific literature review)	PB234-901/AS	A21	34.50
Mutagenicity evaluation of vitamin D_2 (ergocalciferol)	PB81#127-821	A03	7.50
Mutagenicity evaluation of vitamin D_3 (cholecalciferol)	PB81#125-338	A03	7.50

*Price subject to change.

The format of the proposed regulations is different from that in previous GRAS affirmation regulations. The agency has modified the form in which the specific limitations on the use of these ingredients is presented. This change has no substantive effect but is made merely for clarity.

This proposed action does not affect the current use of vitamin D_2 and vitamin D_3 in pet food or animal feed.

REFERENCES

The following information has been placed in the Dockets Management Branch (address above) and may be seen by interested persons from 9 a.m. to 4 p.m., Monday through Friday.

1. "Evaluation of the Health Aspects of Vitamin D_2 and Vitamin D_3 as Food Ingredients," Life Sciences Research Office, Federation of American Societies for Experimental Biology, 9650 Rockville Pike, Bethesda, MD 20014, pp. 7-21, 1978.

2. Ibid., pp. 22-23.

3. Ibid., p. 23.

4. Modderman, J., FDA Memorandum to L. Gosule, "Vitamin D. Estimated Consumption of Vitamin D Through Foods," June 16, 1981.

5. Norman, A. W., Vitamin D: The Calcium Homeostatic Steroid Hormone (Academic Press, 1979).

6. Renwolct, R. E., Letter of September 30, 1981, to L. C. Gosule.

7. Committee on Nutrition of the American Academy of Pediatrics, "Prophylactic Requirements in the Toxicity of Vitamin D," Pediatrics, 31:512, 1963.

8. Peng, S-K., C. B. Taylor, P. Tham, and B. Mikkelson, "Role of Mild Excesses of Vitamin D_3 in Arteriosclerosis. A Study in Squirrel Monkeys," Arterial Wall, 4:229, 1978.

9. Fleischman, A. I., L. Bierenbaum, R. Raichelson, T. Hayton, and P. Watson, in "Atherosclerosis: Proceedings of the Second International Symposium," p. 468, 1969.

10. Lindén, V., "Vitamin D in Myocardial Infarction," British Medical Journal, 3:647, 1974.

11. Peng, S-K. and C. B. Taylor, "Probable Role of Excesses of Vitamin D in Genesis of Arteriosclerosis," Arterial Wall, 6:63, 1980.

12. Gass, G. M. and W. T. Allaben, "Preliminary Report on the Carcinogenic Dose-Response Curve to Oral Vitamin D_2," IRCS Medical Science, 5:477, 1977.

13. Titus, D. S., E. T. Creckman, M. J. Pace, V. R. Klinefelter, and G. M. Gass, "Estrogenic Activity of Oral Vitamin D_2 at Carcinogenic Dose Levels," IRCS Medical Science, 8:286, 1980.

14. Chinn, H. I. (Life Sciences Research Office, Federation of American Societies for Experimental Biology), "A Review of the Adverse Effects of Excessive Intakes of Vitamin D," 1979.

15. Lippe, B., L. Hensen, G. Mendoza, M. Finerman, and M. Welch, "Chronic Vitamin A Intoxication," American Journal of Diseases of Children, 135:634, 1981.

The agency has determined under 21 CFR 25.24(d)(6) (proposed December 11, 1979; 44 FR 71742) that this proposed action is of a type that does not individually or cumulatively have a significant impact on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

FDA, in accordance with the Regulatory Flexibility Act, has considered the effect that this proposal would have on small entities including small businesses and has determined that the effect of this proposal is to maintain current known uses of the

substances covered by this proposal by both large and small businesses. Therefore, FDA certifies in accordance with section 605(b) of the Regulatory Flexibility Act that no significant economic impact on a substantial number of small entities will derive from this action.

In accordance with Executive Order 12291, FDA has carefully analyzed the economic effects of this proposal, and the agency has determined that the rule, if promulgated, will not be a major rule as defined by the Order.

List of Subjects in 21 CFR

Part 182: Generally recognized as safe (GRAS) food ingredients; Spices and flavorings.

Part 184: Direct food ingredients; Food ingredients; Generally recognized as safe (GRAS) food ingredients.

Therefore, under the Federal Food, Drug, and Cosmetic Act (secs. 201(s), 409, 701(a), 52 Stat. 1055, 72 Stat. 1784-1788 as amended (21 U.S.C. 321(s), 348, 371(a))) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10), it is proposed that Parts 182 and 184 be amended as follows:

PART 182--SUBSTANCES GENERALLY RECOGNIZED AS SAFE

1. Part 182 is amended:

§ 182.8950 [Removed]

a. By removing § 182.8950 Vitamin D_2.

§ 182.8953 [Removed]

b. By removing § 182.8953 Vitamin D_3.

PART 184--DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE

2. Part 184 is amended:

a. By adding new § 184.1950, to read as follows:

§ 184.1950 Vitamin D_2.

(a) Vitamin D_2 ($C_{28}H_{44}O$, CAS Reg. No. 50-14-6), also known as ergocalciferol, is the chemical 9,10-seco(5Z,7E,22E)-5,7,10(19),22-ergostatetraene-3β-ol. It is produced by irradiation of ergosterol isolated from yeast and related fungi.

(b) The ingredient meets the specifications of the Food Chemicals Codex, 3d Ed. (1981), p. 344, which is incorporated by reference. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or available for inspection at the Office of the Federal Register, 1100 L St. NW., Washington, DC 20408.

(c)(1) In accordance with § 184.1(b)(2), the ingredient is used in food as the sole source of added vitamin D and is used only within the following specific limitations:

Category of food	Maximum levels in food (as served)	Functional use
Breakfast cereals, § 170.3(n)(4) of this chapter.	350 (IU/100 grams)	Nutrient supplement, § 170.3(o)(20) of this chapter.

Category of food	Maximum levels in food (as served)	Functional use
Grain products and pastas, § 170.3(n)(23) of this chapter.	90 (IU/100 grams)	Do.
Milk, § 170.3(n)(30) of this chapter.	42 (IU/100 grams)	Do.
Milk products, § 170.3(n)(31) of this chapter.	89 (IU/100 grams)	Do.

(2) Vitamin D_2 may be used as the sole source of added vitamin D in infant formula in accordance with section 412(g) of the Federal Food, Drug, and Cosmetic Act (the act) or with regulations promulgated under section 412(a)(2) of the act.

(3) Vitamin D_2 may be used as the sole source of added vitamin D in margarine in accordance with § 166.110 of this chapter.

b. By adding new § 184.1953, to read as follows:

§ 184.1953 Vitamin D_3.

(a) Vitamin D_3 ($C_{27}H_{44}O$, CAS Reg. No. 67-97-0), also known as cholecalciferol, is the chemical 9,10-seco(5Z,7E)-5,7,10(19)-cholestatrien-3-ol. It occurs in, and is isolated from, fish liver oils. It is also the form produced endogenously in humans through sunlight activation of 7-dehydrocholesterol in the skin.

(b) The ingredient meets the specifications of the Food Chemicals Codex, 3d Ed. (1981), p. 345, which is incorporated by reference. Copies are available from the National Academy

Press, 2101 Constitution Ave. NW., Washington, DC 20418, or available for inspection at the Office of the Federal Register, 1100 L St. NW., Washington, DC 20408.

(c)(1) In accordance with § 184.1(b)(2), the ingredient is used in food as the sole source of added vitamin D and is used only within the following specific limitations:

Category of food	Maximum levels in food (as served)	Functional use
Milk, § 170.3(n)(30) of this chapter.	42 (IU/100 grams)	Nutrient supplement, § 170.3(o)(20) of this chapter.
Milk products, § 170.3(n)(31) of this chapter.	89 (IU/100 grams)	Do.

(2) Vitamin D_3 may be used as the sole source of added vitamin D in infant formula in accordance with section 412(g) of the Federal Food, Drug, and Cosmetic Act (the act) or with regulations promulgated under section 412(a)(2) of the act.

(3) Vitamin D_3 may be used as the sole source of added vitamin D in margarine in accordance with § 166.110 of this chapter.

The agency is unaware of any prior sanction for use of these ingredients in foods under conditions different from those identified in this document. Any person who intends to assert or rely on such a sanction shall submit proof of its existence in response to this proposal. The action proposed above will constitute a determination that excluded uses would result in

adulteration of the food in violation of section 402 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 342), and the failure of any person to come forward wth proof of an applicable prior sanction in response to this proposal constitutes a waiver of the right to assert or rely on it later. Should any person submit proof of the existence of a prior sanction, the agency hereby proposes to recognize such use by issuing an appropriate regulation under Part 181 (21 CFR Part 181) or affirming it as GRAS under Part 184 or 186 (21 CFR Part 184 or 186), as appropriate.

Interested persons may, on or before (insert date 60 days after date of publication in the FEDERAL REGISTER) submit to the Dockets Management Branch (address above) written comments regarding this proposal. Two copies of any comments are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. Received comments may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

Dated: March 31, 1983.

MAR 31 1983

William F. Randolph

William F. Randolph
Acting Associate Commissioner for
Regulatory Affairs

CERTIFIED TO BE A TRUE COPY OF THE ORIGINAL

Dana G. Thomas